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MICROBIAL CONTROL—THE EMERGENCE OF AN IDEA

A Brief History of Insect Pathology Through the Nineteenth Century[1]

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INTRODUCTION

THE TERM "microbial control" is used in referring to that phase of biological control concerned with the employment by man of microorganisms for the control and reduction of the number of animals (or plants) in a particular area or in a given population. As it applies to insect populations, it thus distinguishes between the use of microorganisms and the use of insect parasites and predators.[3]

Although the term "microbial control" is a modern one—first used in 1949 (Steinhaus, 1949)[4]—the idea it designates originated over a hundred years ago. Unfortunately, the manner in which this idea was born and just how it emerged from the morass of early biological thought and experiment have never been made clear. Almost universally the literature in insect pathology credits the illustrious zoologist Elie Metchnikoff[5] with having first suggested using microorganisms to control harmful insects. Metchnikoff's contributions in this field were great, but he was not the first to propound the idea, and the story is much involved.

Also not generally appreciated is the fact that the idea of microbial control was conceived and brought forth largely as a result of man's study of the maladies of the silkworm, *Bombyx mori* Linnaeus. Just how this all came about makes a fascinating and revealing study, and one that can contribute

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[3] Most authorities distinguish between "biological control" (use of organisms by man) and "natural control" (an effect of organisms, as well as other factors, as they occur in nature). The arbitrary designation of "microbial control" as a type of "biological control" is dictated largely by the desire for a convenient and succinct term that conveys the idea of man using microorganisms (including viruses) to control noxious pests, especially insects.

[4] See Literature Cited for citations referred to in text by author and date.

[5] An alphabetical index to the proper names referred to in this publication may be found at the end of the paper.

significantly to our better understanding of the concept of microbial control and its future possibilities.

The purpose of the present paper is to trace the birth and growth of the idea of microbial control (one kind of applied insect pathology) through the earliest years of its history, while at the same time presenting, as a background, the development of the field of insect pathology prior to and during this period. I have arbitrarily chosen to limit this brief historical treatment to the years preceding 1900. Even so, only the salient features of the history of insect pathology up through the nineteenth century will be presented, and these without any claim to completeness or to certainty of interpretation. They are offered merely with the hope that they will be helpful to a better understanding of the heritage of insect pathology and microbial control. As affirmed by Aristotle in his *Politics:* "Here and elsewhere we shall not obtain the best insight into things until we actually see them growing from the beginning. . . ."

EARLY BASIC SCIENTIFIC OBSERVATIONS

Earliest References to Diseases in Insects

Some insects suffering from disease are so spectacularly affected that even primitive man must have given them a second glance, and perhaps likened their condition to that of larger animals suffering a deadly malady. However, we know of no cave-wall art, no etchings or symbols on stone, and no archeological artifacts that would lead us to believe that early man was particularly conscious of the phenomenon of disease among the invertebrates in his environment. We may, perhaps, be permitted to speculate that man's attention to diseases in insects was focused first on the maladies of the silkworm and the honey bee. We can imagine, for example, that Empress See-ling-shee (Si-ling-chi), "the first and legitimate" consort of Emperor Hoang-tee (Hoangti), noticed diseased or abnormal silkworms among those she tended with much care in the Imperial apartments. This historical beginning of practical sericulture occurred about 2700 years before the time of Christ.

As with many other aspects of historical biology, we find the first known recorded mention of disease and abnormalities in insects in the writings of Aristotle (B.C. 384–322). In his *Historia Animalium* (probably written between 335 and 322 B.C.) he tells of the destruction of honeycombs by what we know today to be the wax moth, which he further accuses of "bringing disease into the swarm." He observes that bees "suffer most" when flowers are covered with mildew, or in seasons of drought. Other disease conditions of bees recorded by Aristotle include a lassitude, a "dispirited" condition, hunger, and what we may interpret as one of the foulbroods. He generalizes that all insects die if they are smeared over with oil. Another early naturalist and author, Pliny (A.D. 23–79), also referred to afflictions of bees in his writings on natural history (A.D. 77), highlighting the "grief" experienced by these insects when one of their "kings should happen to be carried off by the pestilence." The poet Virgil, in his *Georgics* (B.C. 37–29), begins a charmingly anthropomorphic description of a disease of bees with the lines: "Since life brings to the bees the same bad luck as to humans, They may suffer severe

illness. . . ." Moreover, he recommends a fascinating "tonic food" to aid the bees in regaining their health.

It is reasonable to suppose that as sericulture became one of the major industries of the Orient, the diseases of the silkworm increased in their incidence and general importance.[6] It would be impossible to say whether or not some of the pathogens of this insect accompanied those first eggs brought to Constantinople, about the year 555, in the hollow pilgrim staffs of the two monks who risked death to bring the secrets of silkworm rearing to the West. Many students of sericulture believe that the disease now designated as muscardine was known about 1000 A.D. In any case, we know that eventually disease became a serious problem in European sericulture. Silkworm maladies even gained sufficient notoriety to be celebrated in poetry. In the year 1527, Marcus Hieronymus Vida published his poem on the silkworm ("De Bombicum"), and included a surprisingly long passage on the diseases of the insect. The poem has been translated (in 1750) from the original Latin into English by the Reverend Samuel Pullein of Trinity College, Dublin. The passage pertaining to disease begins:

> Come learn what healing helps should be prepar'd
> When dire diseases threat the sick'ning herd:
> Like us attack'd, their tender bodies know
> Mortal mischance, and feel their lot of woe;
> Pale sickness shakes alike their little frames;
> Whether the tainted air's corrupting steams
> Or noxious food the latent poison hold,
> Whate'er the cause, infection thins the fold;
> Fate triumphs, bodies stain'd with putrid gore
> Deform the shelves and fun'rals strow the floor;
> No flatt'ring prospect heaps the golden thread,
> But ev'ry hope lies mingled with the dead.

The Italian poet goes on to describe the diseases and to recommend procedures by which they can be avoided or eliminated.

During the sixteenth, seventeenth, and eighteenth centuries, the matter of

[6] The author would like to express his regret that he is unable to include in this paper significant historical facts pertaining to the early history of silkworm diseases in the Orient. It is not unlikely that the Chinese and Japanese, for instance, made interesting observations that would enrich any presentation of the early history of insect pathology. As indicated by Ishikawa's (1940) book, "Pathology of the Silk-Worm," however, not a great deal of critical scientific work was done in this part of the world prior to 1900. Nevertheless, interesting factual descriptions and narratives have been recorded. For example, Ishikawa refers to an 1812 report by Narita who tells of the use of muscardined silkworms as a medicinal in the treatment of palsy or paralysis as early as 900 A.D. A code of laws compiled between 905 and 927 A.D. ordered that each year a certain quantity of muscardined silkworms had to be delivered from eleven different provinces of Japan to the bureau of medicine to be used for the treatment of palsy. Ishikawa also refers to the fact that an early book on sericulture "Kaiko-shiyoho-ki," written in 1702 by Dogen Nomoto, contained a chapter dealing with the diseases of the silkworm. Other eighteenth-century books, all containing descriptions of silkworm maladies, were published by other Japanese authors (Baba, Tsukada, and Sato) in 1712, 1755, and 1766, respectively. Robinet (1843) reviews some of the observations of early Chinese authors, as gleaned from an 1837 work by Stanislaus Julien.

disease increasingly came to be included in writings on the techniques of sericulture.[7] In 1830, Count Dandolo declared, in a chapter on disease in a book on the rearing of the silkworm, that "hundreds of works" had been written on the diseases of this insect. Mention of the subject also occurred in general works that included discussions of the silkworm, such as a book written on butterflies in 1679 by Maria Sibylla Merian. Published works on sericulture became more and more scientific in character and the need for a more factual comprehension of the diseases involved became clear. It is noteworthy that as the concept of scientific method developed and biological science began to emerge into the realm of experimental science, among the first problems to gain the attention of biological investigators were those pertaining to the diseases of the silkworm. The significance of these studies from the standpoint of their influence on the understanding of human disease and such concepts as spontaneous generation is great. One of the earliest of what might be called a scientific consideration of the diseases of the silkworm appeared in 1808. This was a treatise, "Recherches sur les Maladies des Vers à Soie," by P. H. Nysten who became an oft-quoted authority on the subject during the years to follow. Among other early writers whose works included discussion of diseases of the silkworm were Boissier (1763), Pomier (1763), Aymard (1793), Montagne (1836), Audouin (1837 *a,b,c*), Robinet (1843)—(who, incidentally, refers to such smaller works as those by Bérard, Carrier, Raynaud, Robert, Vincens de Saint Laurent, and others)—Robin (1847, 1853), Guérin-Méneville (1847, 1849), Maestri (1856), Cornalia (1856), Lebert (1858), and de Quatrefages (1859). Masera (1956*b*) tells us that the first to mention the disease we know as muscardine in the sericulture circles of Europe was Antonio Vallisnieri (or Valisneri, as he signed his name) who, around 1708 and 1710, advanced certain ideas as to its cause.

Although disease as an abnormal condition in insects was in all probability first critically observed in the honey bee and silkworm, the first microbial parasite was not seen in either of these insects. As might be expected, because of their macroscopic as well as microscopic appearance, the first forms of microbial life found associated with insects were fungi—or "vegetable growths" as some of the early writers referred to them. The first published record appears to be one concerned with the "Chinese plant worm" (Hia Tsao Tom Tchom), mentioned as early as 1726 by de Reaumur and in 1736 by Du Halde. De Reaumur, in effect, relayed a report by a Jesuit priest named Parennin who sent specimens of it from the Orient to France. This "worm" (apparently the larva of a species of noctuid, probably an *Agrotis*) consisted of a larva from which emerged a stemlike vegetable growth (fig. 1) characteristic of the group of fungi now designated by the generic name *Cordyceps*. The species concerned in this instance appears to have been *Cordyceps sinensis* Berkeley. The so-called Chinese plant worm was considered to be of great value as a rare drug, and in China was used by the emperor's

[7] American readers may be interested to know that brief references to disease are contained in a very interesting document importuning the settlers in Virginia to raise silkworms. The tract is dated 1655 and was printed in London by John Streater for Giles Calvert. It is titled "The reformed Virginia silk-worm, or, a rare and new discovery of a speedy way, and easie means, found out by a young Lady in *England*, she having made full proof thereof in *May, Anno* 1652."

physicians. (The writer has found it still possible to purchase *Cordyceps*-infected larvae in herb stores in San Francisco's Chinatown.) A rather romantic history is associated with the fungus concerned, and for an early account of it the reader is referred to Cooke (1892).

Another early, or perhaps even earlier, record of an entomogenous fungus was that made by Christian Paulinus in the beginning of the eighteenth century when he wrote that "certain trees in the island of Sombrero in the East

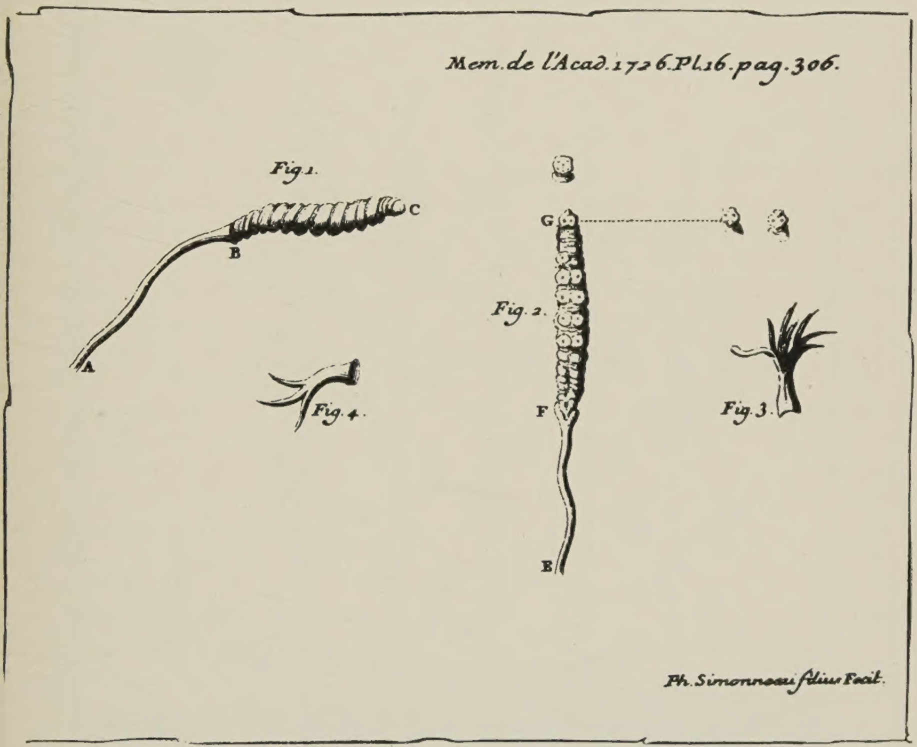

Fig. 1. Reproduction of what is apparently the first published illustration of a diseased insect. The drawings, representing *Cordyceps*-infected larvae, accompanied a paper by R.-A. de Reaumur published in 1726 (Mém. Acad. Roy. Sci., pp. 302–05.)

Indies have large worms attached to them underground, in the place of roots" (see Gray, 1858, who cites numerous other early authors and observers of *Cordyceps*-infected insects.) Undoubtedly he was alluding to an instance of *Cordyceps* infection.

Insects parasitized by *Cordyceps* fungi were frequently known as "vegetable wasps" and "plant worms" (or "awetos" in New Zealand). Among the most celebrated of the "vegetable wasps" were those (*Vespa* and *Polybia*) infected with *Cordyceps sphecocephala* (Klotzsch). In an account titled "Apparato para la historia natural de España," Torrubia, a Franciscan friar, tells of finding, in 1749, some dead wasps in a field near Havana:

> One day, I found in the fields several dead wasps with their skeletons and wings intact, and out of their bellies were growing little trees which

Fig. 2. Reproduction of a plate from a publication by J. Torrubia, published in 1754. It represents dead wasps with "trees" (*Cordyceps*) growing from them. The three individuals at the top of the illustration are probably not meant to represent wasps in flight, but were simply placed there because of the clear space provided. The plants shown in the center of the figure undoubtedly represent further developed "trees."

> when fully grown attain a size of up to five hands. This plant is called *Gia* by the inhabitants of these regions, and it is covered with very sharp thorns. The natives attribute these thorns to the wasps' bellies which produced the plant: for this reason, they say, the plant is covered with stingers.
>
> The existence of this shrub was not commonly known until I made it public. After careful observations which I made with a microscope, I sent with a young man called Centellas a dead wasp, perfectly preserved, with a rather fair sized tree growing from it, to the Treasurer of my Order and my principal benefactor, Señor don Martín de Arostegui. . . .

Torrubia gives a diagrammatic representation (fig. 2) of two wasps lying on the ground with a "tree" growing out of the base of each abdomen, while three other wasps, each having a similar tree affixed to it, are drawn above similar plants. In all probability these "trees" represent species of *Cordyceps.* Torrubia concludes his report with an entertaining poem about his findings. He wrote the poem to accompany the specimen which he sent to de Arostegui.

It cannot be assumed that the earliest observers of *Cordyceps* on insects realized that they were concerned with instances of infectious disease. Some of them probably accepted the concept of the Chinese philosophers that the infected specimens were herbs during the summer, changing into "worms" when winter appeared. Others considered them to be plants which simply looked like "worms," and still others thought they represented "worms" that had merely attached themselves to the plants or their roots. Nevertheless, one cannot escape the feeling that by the end of the eighteenth century the parasitic essence of the relationship between plant and insect was in some vague way appreciated. As early as 1769, Fougeroux affirmed that the plant "presses and takes hold" of the insect's body, attaching itself to it. In any case, it is worth our remembering that the first fungi reported in association with insects were pathogenic forms. (Saprophytic species growing on the bodies of insects immersed in water were first reported in 1760 [Ledermüller, 1760].) Most of these and other early reports on entomogenous fungi have been cited by Robin (1847), Gray (1858), and Cooke (1892), and their writings should be consulted by the reader interested in the nature of these early but fascinating accounts. Of particular interest to the insect pathologist, for example, is the generalized description of the pathogenesis of fungus infections in insects as presented by Gray toward the end of his pamphlet on "fungoid parasites" of insects. Incidentally, one of the earliest American accounts of a *Cordyceps* infection appears to be that by Cist in 1824. He reported the fungus on a *Melolantha*—a cockchafer.

Some of the early leading naturalists recorded the occurrence of disease in insects which they were observing for taxonomic purposes. For example, in 1776, De Geer published probably the first description of what we now know to be an *Empusa* infection in flies; he thought that the flies may have eaten poisonous food. De Geer accompanies his description with a figure in the legend of which he lists as victims of the disease the "Honigfliege (*M. mielleuse*)," and the "Haus- und Stubenfliegen." In the 1782 German edition

of De Geer's work, the translator, Goeze, appends a footnote in which he refers to observations by Winterschmidts, in 1765, on a disease of mites (*"Milben"*) that often kills thousands of the arthropods. Latreille (1805), another early naturalist, also refers to a disease of domestic flies that in all probability was caused by an *Empusa* fungus. In no real sense, however, was the microbial nature of these diseases realized.

The Account by William Kirby

Shortly after the turn of the nineteenth century there appeared the first distinctive writing on the diseases of insects in general that might be considered to be in any sense comprehensive. This rather remarkable presentation appeared in 1826 as a chapter (Letter XLIV) in volume 4 of the notable treatise, "An Introduction to Entomology," by Kirby and Spence. This particular chapter, titled "Diseases of Insects," was written by Kirby (fig. 3) who, in his opening remarks, dutifully explained why he was assigning an entire and separate chapter to a consideration of the diseases of insects. A brief review of this chapter is eminently worth while here since it so well portrays the concepts of disease in insects as generally held by biologists of that time.

Kirby divides the diseases of insects into two large classes: Those resulting from "some accidental *external* injury or *internal* derangement, and those produced by *parasitic* assailants." Under the first of these designations he discusses wounds, fractures, mutilations, tumors, and monstrosities. Diseases resulting from an internal cause included those described as a "kind of vertigo" (believed to result from a derangement of the nervous system), a "kind of convulsions," "the stone" or calculus, and what we now know to be some of the various infectious diseases. Had he realized the infectious or parasitic nature of the latter group, Kirby would have undoubtedly placed them with the second large class of maladies, i.e., those caused by "parasitic assailants." In this group Kirby included those diseases caused by some of the more obvious fungi, nematodes, and parasitic insects. Entomophagous insects are considered in some detail, and the results of their activities are considered to represent, and properly so, a diseased state.

It is clear that by the time Kirby wrote his chapter on disease, insects mortally affected by entomogenous fungi were being observed frequently and described as resulting from a variety of causes. We have already mentioned the records of De Geer and of Latreille in this respect. Kirby himself clearly describes an affliction of Diptera that undoubtedly was caused by an entomophthoraceous fungus. That a fungus was involved in these cases was not, however, suspected by Kirby who believed the disease arose "from a superabundance of the nutritive fluid, or of the fat, so that it seems to be a kind of *plethora*." On the other hand, Kirby, as did Persoon and others before him, recognized the fact that true fungi did grow upon the larvae and pupae of some insects. He sagaciously differentiated between the saprophytic growth of fungi on the body of dead insects (such as first observed by Ledermüller, 1760), and the possibility of fungi growing at the expense of living tissue. He points out that Persoon (1801) did not make it clear whether or not the insects on which he reported two species of *Isaria* to grow were dead

or alive. If alive, Kirby suggests that "perhaps in these cases these plants may constitute an insect disease." A remarkable deduction for that day—and possibly derived from the fact that as early as 1799 Kirby had interested himself in and had published on fungi parasitic on certain grain plants.

Kirby's discussion includes a brief consideration of the diseases of the honey bee and the silkworm. The cause of these diseases he attributes to

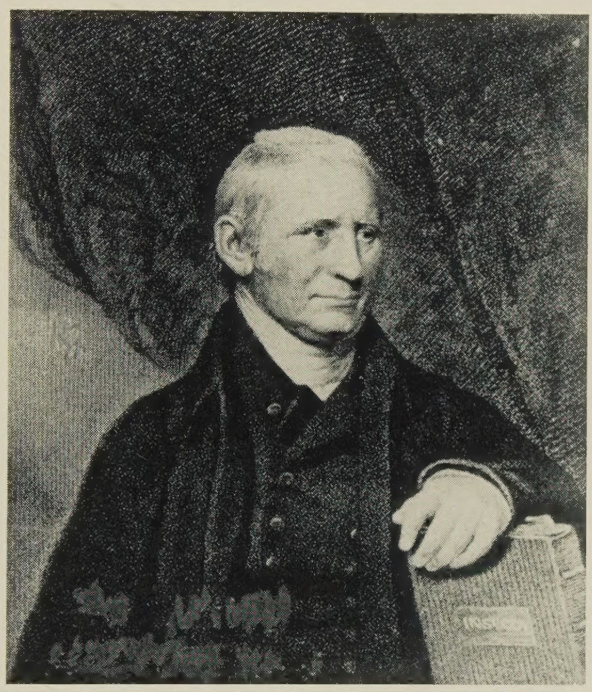

Fig. 3. William Kirby (1759–1850), English clergyman and entomologist who wrote one of the first comprehensive accounts of diseases affecting insects. This account was published as Chapter XLIV in "An Introduction to Entomology" by W. Kirby and W. Spence (1826). (Reproduced from an illustration in J. Freeman's (1852) "Life of the Reverend William Kirby.")

poisonous food or mephitic and other types of noxious air. He ends the chapter with a very interesting account of the infection of insects with worms, such as *Gordius,* and recounts some of the early observations by De Geer and others along this line. One is impressed with the surprisingly advanced state of knowledge existing at that time relative to nematode infections in insects.[8]

The Contributions of Agostino Bassi

It was during the nineteenth century that certain maladies of the silkworm were destined to play an important role in man's understanding of infectious disease. Naturally, these same advances were momentous and highly signifi-

[8] An exchange of correspondence between Kirby and Thomas Brightwell relating to the ingestion of *Gordius* by a beetle (*Harpalus*) is recorded (p. 418) in a biography of Kirby by John Freeman (1852).

Fig. 4. Agostino Bassi (1773–1856), pioneer insect pathologist, first demonstrated the parasitic nature of muscardine disease of the silkworm. Many regard him as the founder of the doctrine of pathogenic microbes. His great work, *Del Mal del Segno, Calcinaccio o Moscardino,* was published in 1835.

cant in the development of insect pathology. As early as 1546 Fracastoro (and two hundred years later Plenciz), without any experimental proof, had propounded theories that diseases were caused by seeds of infection. However, the idea of spontaneous generation, that is, of the generation of life out of dead and inert matter, remained a popular conception until finally overthrown in the latter part of the nineteenth century. Among those whose work not only aided in overthrowing the theory of spontaneous generation but helped to establish the germ theory of disease was Agostino Bassi (1773-1856) (fig. 4), sometimes referred to as Bassi de Lodi after the town in northern Italy where he did his work and lived most of his life. The importance of Bassi's work has to a great extent been overshadowed by that of Pasteur's a few years later, but his genius deserves greater recognition than it has received. It was Bassi whose discoveries ushered in the dawn of the science of infectious disease. Certainly he stands as a giant among those early workers who may be said to have laid the first foundations for insect pathology.

Around 1800, in Italy as well as in France, a disease known as "mal del segno" or "calcino" (or, in France, "muscardine") began to reach serious proportions in silkworm *ménageries*. This was a time when the matter of contagion was being given considerable philosophical and medical attention. It is not surprising, therefore, that the contagious nature of this disease of silkworms was recognized even though the cause of the disease was unknown, and it was thought, by many, to arise *de novo*. In 1821, Foscarini reported on a series of experiments by which he was able to show that silkworms dead of the disease, as well as tools used in handling them, were infectious, and that the infectiousness could be destroyed by passing the tools through, or repeatedly over, a flame. Foscarini, however, did not recognize the true cause of the disease; he considered the cause to be a contagious miasma. It remained for Bassi and others to clarify this basic mystery.

With remarkable ingenuity Bassi showed that the disease is caused by a vegetable parasite (the fungus we know today as *Beauveria bassiana* (Balsamo)),[9] that this parasite grows and develops in the living silkworm, that it eventually causes the insect's death, that the diseased insect is, by virtue of the parasite's presence, rendered infectious, and that the infectious agent could be transmitted by inoculation, by contact, and by contaminated food. The characteristic white covering, or efflorescence, of the diseased larvae he showed to consist of but a mass of the causative fungus. He explained how the "seeds" conceivably produced by this mass were readily disseminated and how they were responsible for the disease in new individuals. He correctly

[9] Bassi, handicapped by failing vision and a lack of training in cryptogamic botany, gained the assistance of Giuseppe Balsamo Crivelli in determining the nature of the fungus. Balsamo (1835) placed the organism in the genus *Botrytis* and first named it *Botrytis paradoxa*. Later he changed the name to *Botrytis bassiana* in honor of its discoverer. A still later (1912) revision by Vuillemin made the fungus the type species of the genus *Beauveria*.

It might be pointed out that according to Robin (1853), a paper by Lomeni appeared in 1835 claiming that before the reports of Bassi and Balsamo, muscardine was known to be caused by a fungus, and that certain other assertions by Bassi lacked proof. Examination of records and published reports prior to those of Bassi, however, do not justify these retrospective claims by Lomeni.

ascertained that warm, humid conditions facilitated the growth and development of the fungus. He demonstrated that the pathogen can be destroyed by certain chemical and physical means. Indeed, his recommendations for controlling and preventing the disease (through disinfection with lye, wine, boiling, burning, and exposure to sunlight) were of such an advanced caliber that he might also be considered as one of the founders of modern disinfection. Thus it was Bassi who, for the first time, showed experimentally that a microorganism (the fungus) was the cause of an infectious disease in an animal (the silkworm)! He first presented his findings in 1834 in a communication delivered before a commission of the Faculties of Medicine and Philosophy of the University of Pavia. For this accomplishment he is regarded by many as the founder of the doctrine of pathogenic microbes or the germ-theory of disease. It is true that some time earlier Nysten (1808), upon microscopic examination, saw the fungus associated with the dead silkworms, but he did not associate it with the cause of the disease which he recognized as being of a contagious nature but which he believed to be the result of certain chemical changes in the afflicted insects. It is the glory of Bassi's work that his findings were based on sound experimentation. (In a later section we shall emphasize Bassi's contribution to the idea of microbial control of noxious insects.)

Bassi was, in many ways, a remarkable and romantic man. He was born in 1773, in Mairago, near Lodi. He was educated in law, but also studied natural science in Pavia. Although from time to time he held various civil posts, and was invited to fill others, he gave up most of them because of failing eyesight and general ill health. In dire financial straits, he turned for a living to farming and the raising of sheep—an unsuccessful venture except that it induced him to write informative articles on potatoes, cheese, wine, and sheep raising. Fortunately, through the inheritance of a legacy from a relative, he was able to discharge his debts and to give more attention to scientific pursuits. The threat of calcino (muscardine) to the sericulture industry made him determined to find means of preventing the disease. His studies on the disease covered a period of about twenty-five years, and out of them came his great work, *Del Mal del Segno Calcinaccio o Moscardino,* published in Lodi in 1835 and 1836. From this study, Bassi proceeded to extrapolate his findings and thinking into the field of human diseases. He wrote noteworthy treatises on contagion (1844), pellagra (1846), and cholera (1849). He became totally blind in 1856, the year in which he died. Before his death, extensive recognition and many honors came to him. After his death, much of his work was generally forgotten, but in 1901 the city of Lodi transferred his ashes to a new cemetery, named a street in his honor, and designated his old home with a plaque. Italian scientific societies have since honored him in various ways, including the reprinting of many of his published works. The Sixth International Congress for Microbiology (Rome, 1953) was honored by an Italian stamp portraying Bassi and his work with the silkworm. An occasional paper (e.g., Major, 1944; Ainsworth, 1956; Masera, 1956*a*, *b*) properly attests to the significance of Bassi's work. Nevertheless, most writers of treatises and textbooks on infectious disease have yet to accord him the honor and credit he deserves.

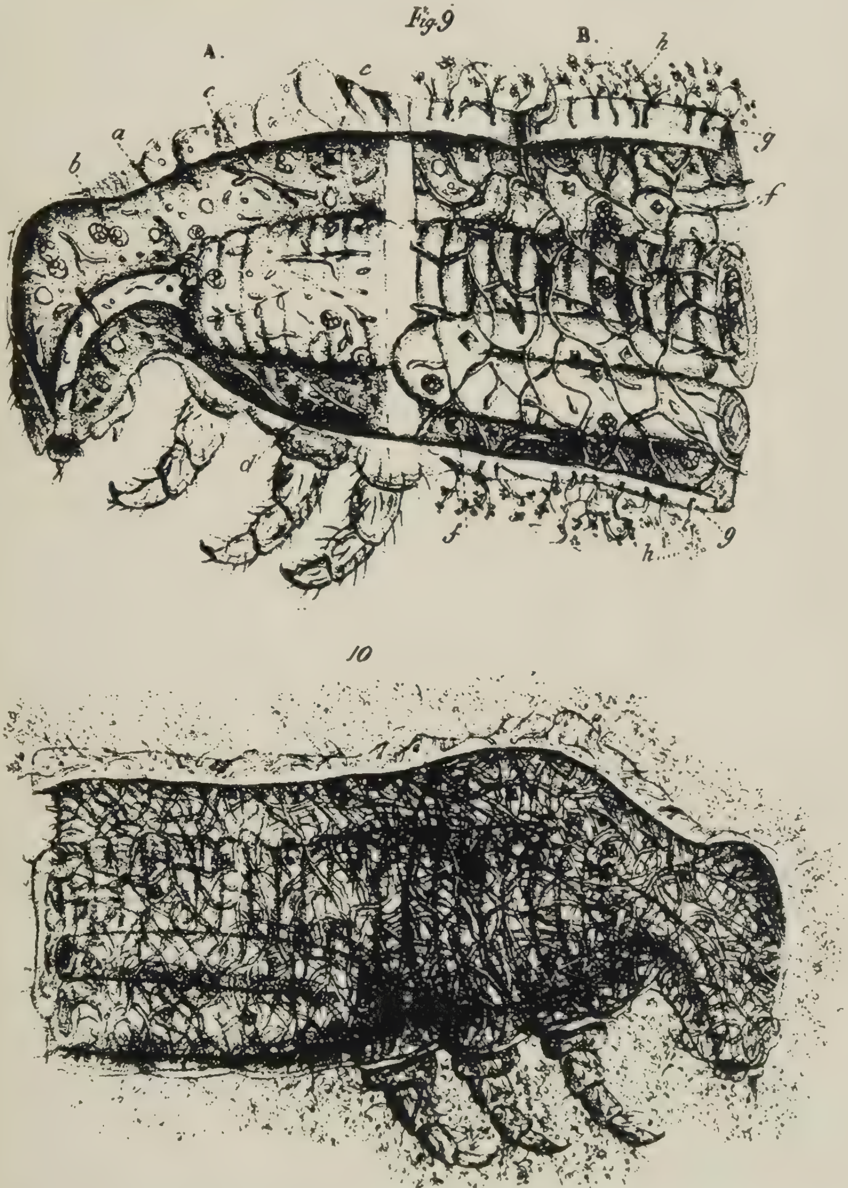

Fig. 5. An early representation of a muscardined silkworm showing the nature of the infection by the fungus *Beauveria bassiana* (Bals.). (From Maestri, 1856.)

It must be admitted that at first Bassi's findings relative to the "mal del segno" were not universally accepted. They were especially attacked by the proponents of the theory of spontaneous generation, and by others who acknowledged the presence of the fungus in the diseased larvae but who did not believe that it in itself could cause the disease. Eventually, however, the skepticism gradually was overcome, especially after 1851 when Vittadini described the spores of the fungus, isolated and cultivated the fungus on nonliving media, and demonstrated conclusively that it was the specific and only cause of the disease. Thus Bassi joined the ranks of those like Redi, Spallanzani, and others, who set the stage so that the great Pasteur could finally, in 1860–1861, deal the death blow to the ancient belief of spontaneous generation, and John Tyndall, a few years later, could lay its ghost. Bassi's achievements in this regard are marred only by the fact that in later years he had some misgivings about his stand against spontaneous generation, and apparently believed it to be possible under certain conditions.

The Contributions of Louis Pasteur

Like Bassi, Louis Pasteur (1822-1895) approached and entered the field of microbial disease and pathology in animals by studying the diseases of the silkworm. The diseases studied by Pasteur, however, did not include the fungus infection elucidated by Bassi, but instead were concerned with maladies caused by protozoa and bacteria. Although he knew of Bassi's work, and of Audouin's (1837 *a,b*) confirmation of it, there is no indication that Pasteur's experimentation was significantly affected by that of the Italian's. Nevertheless, Pasteur did have a background of observations by many sericulturists and biologists from which he profited and against which he could project his own discoveries.

The breeding of silkworms for the manufacture of silk began in France during the thirteenth and fourteenth centuries. During the first half of the nineteenth century sericulture flourished at an accelerated rate, annual production rising from 6 million kilograms in 1788 to 26 million kilograms in 1853—about one tenth of the world's production. Unfortunately for France, this progressive increase in production did not continue without serious interruption. About the middle of the nineteenth century there appeared throughout southern France a combination of devastating plagues of the silkworms that threatened the entire silk industry. Production fell to but 4 million kilograms per year by 1865. Disease-free eggs ("seed") of the insect could no longer be produced in France, but had to be imported. The diseases spread to Italy, Spain, and Austria, then to Greece, Turkey, and the Caucasus, and eventually to the Orient. The spreading diseases, of course, further restricted the source of safe eggs.

In desperation, 3,574 owners of silkworm nurseries petitioned (in 1865) the government of France to cope with the disastrous pestilence. They further requested that measures be taken to reduce taxes, to supply silkworm breeders with reliable strains of eggs, and to provide for a study of "all questions related to this persistent epizootic, as much from the point of view of pathology as from that of hygiene." This petition reached the Senate where the deliberations were led by J. B. Dumas, friend and former teacher of Pasteur.

While preparing his report for the Senate, Dumas persuasively asked Pasteur to undertake the investigation requested by the petitioners. After professing some apprehension over his ignorance of silkworms and sericulture, Pasteur accepted the challenge, probably largely out of respect for his master, but also he felt that the investigation might come within the range of his studies on fermentation and the "diseases" of wines.

Pasteur left Paris on June 6, 1865, going directly to the Department of Gard, the center of the area where the diseases reigned in greatest intensity. Here, in the town of Alès (Alais), he began his investigations, making numerous general observations of the nurseries, and interviewing silkworm breeders. In September he submitted a report of his observations to the Academy of Sciences. The situation was a discouraging one; truly a catastrophe had struck this leading agricultural pursuit of southern France. To one with lesser confidence in himself than that which Pasteur possessed, the matter might have appeared insoluble. Skeptical breeders were obviously disappointed that the government would send a "mere chemist" to investigate their trouble. The death of his father and of his youngest daughter at this time added to Pasteur's problems. Nevertheless, in 1866 he returned to Alès with two assistants, Gernez and Maillot (and later Duclaux), and, in a lonely house a short distance out of town, established a laboratory at Pont Gisquet at the foot of the Mount of the Hermitage (fig. 6). Here, burdened with the death of another of his daughters, he began the intensive investigations that were not only to save the silk industry of France, but to add one of the most brilliant chapters to man's understanding of infectious processes and to the scientific development of insect pathology!

At the time Pasteur began his experiments, a clear distinction was not made between two of the diseases afflicting the silkworm. Pasteur himself did not recognize the differences until his studies had proceeded for about two years when he differentiated the disease known as "pebrine" (French *pébrine*) from that called "flacherie." Pebrine derives its name from the small spots resembling grains of black pepper on the integument of diseased caterpillars. It is characterized by the presence, in the tissues of the diseased insect, of numerous small oval spores which were called "corpuscles" throughout the early literature. Before Pasteur began his work, these spores, or corpuscles, had been observed by such men as Guérin-Méneville (1849) (who called them "hematozoïdes"), De Filippi (1851, 1852), Cornalia (1856) (fig. 7), Lebert and Frey (1856) (who considered them to represent a vegetable parasite which Lebert (1858) named *Panhistophyton ovatum*), Naegeli (1857) (who, believing them to be a yeastlike fungus, gave to them the present name of *Nosema bombycis*), Osimo (1859), and de Quatrefages (1859). Brouzet (1863) compared the corpuscles with the animalcules seen by Rayer and Davaine in the blood of sheep dead of anthrax, and predicted that pebrine would be successfully combated when a means of destroying the corpuscles was found. Béchamp[10] (1867) correctly believed the corpuscles to be the spores

[10] Antoine Béchamp was an implacable opponent of Pasteur with whom he differed on a number of issues, including the germ theory and the nature of the silkworm diseases. He formulated a doctrine of microzymas, microscopic granules he believed to be the basis of life (see Bulloch, 1938). In the case of pebrine of the silkworm, however, it appears that Béchamp appreciated the parasitic nature of the disease before Pasteur did, even though

Fig. 6. The site of Louis Pasteur's laboratory at Pont Gisquet, near Alès, France. (A) The house (in the foreground) is that in which Pasteur accomplished his work on pebrine and flacherie of the silkworm. (From Pasteur, 1870.) (B) Pasteur, by the Pont Gisquet laboratory, dictating a scientific paper to his wife. (Photo courtesy Dr. Rene J. Dubos.) (C) Street-side view of laboratory as it appears today. Inset shows close-up of commemorative plaque on outside wall of laboratory, now a private residence. (Photo courtesy M. E. Martignoni.) (D) Statue (by Tony-Noel in 1896) of Pasteur in public square in Alès. Allegorically, Pasteur is helping the silk industry (according to A. Schenk, a "magnanarelle" or woman who rears silkworms) to her feet. (Original photo.)

of a parasitic microorganism. Today, following the observations of Balbiani (1882) and Stempell (1909), the corpuscles are recognized as a stage (the spore) in the life cycle of a protozoan in the order Microsporidia of the class Sporozoa.

In spite of the earlier suggestions as to the parasitic nature of the corpuscles, Pasteur, strangely enough, approached the problem unwilling to accept the idea. Whether or not his opinions were affected by his chemical background or by such men as Chavannes (1862) who thought pebrine to be caused by abnormal metabolic changes is not known. In any case, for two years he believed that the disease was caused primarily by physiological disturbances, and that the corpuscles were merely products of tissue disintegration. Eventually, however, Pasteur's assistants became convinced that the corpuscles were the cause of the disease, and subsequently Pasteur himself reached the same conclusion, recognizing their relation to other "psorosperms" of that day being described by Leydig (1853) and others in other invertebrates.

Of great importance was Pasteur's observation, like those of Osimo (1859) and Vittadini (1859) before him, that the pathogen could be transmitted through the egg of the insect, as well as by contact with diseased silkworms, and through the ingestion of contaminated food. On the basis of this information he was able to select eggs which gave rise to healthy larvae. If, by microscopic examination, the moth that laid a given batch of eggs was found to harbor corpuscles of the disease, the eggs and moth were both destroyed by burning. On the other hand, eggs from moths showing no corpuscles in their tissues would yield silkworms free of pebrine. At first, skeptical sericulturists disdained the idea of the microscope being an effective tool in the control of the disease. To this Pasteur retorted, "There is in my laboratory a little girl [his daughter, Marie-Louise] eight years of age who has learned to use it without difficulty." (And we may here interpose the thought that Marie-Louise was the forerunner of our modern medical technician. Also, Pasteur's was apparently the first laboratory to employ a microscope for the diagnoses of infectious diseases.)

The early skepticism of Pasteur's method (with due credit to the proposals along this line, in 1859, by Osimo and Vittadini) gradually dissipated as small lots of selected eggs distributed among producers almost invariably gave rise to pebrine-free silkworms. Added to this convincing proof, was the energetic "campaign" by Pasteur who through enormous amounts of correspondence, articles in trade journals, and scientific papers finally subdued virtually all criticism, and won the approval of the government including the applause of his friend Dumas.

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they began their researches on the malady at about the same time. Despite rather fantastic claims made for Béchamp's work by one writer (Hume, 1923), however, Béchamp never really mastered the problem in all its facets. Certain details of his observations and conclusions were in error, so that in the end Pasteur's understanding of the disease and its control rested on a more firm and lasting scientific, as well as practical, foundation. In the case of the malady known as *flacherie*, Béchamp resorted to his microzyma theory, considering the disease to be caused by an abnormal development of the microzymas in the body cells of the silkworm.

We have mentioned that Pasteur, in his work at Alès, was concerned with at least two diseases of the silkworm. Pasteur realized this when he found larvae free of pebrine corpuscles but dying in a soft, flaccid condition, and becoming black and decayed. This disease was called *morts-flats,* and later *flacherie.* At first its presence discouraged Pasteur because of the confusion it caused—"Nothing is accomplished; there are two diseases!" he complained to his assistants.

Gradually, differentiation of the two diseases became easier, especially when Pasteur was able to associate the presence of certain bacteria with the flaccid condition. The disease occurred when these bacteria multiplied in large numbers in the digestive tract of the silkworm. One of these bacteria was a coccus arranged more or less in chains, which Pasteur spoke of as *"ferment en chapelets de grains,"* and which today is known as *Streptococcus bombycis* (Béchamp). The other was a sporeforming bacillus, Pasteur's *"vibrion à noyau,"* now known as *Bacillus bombycis* auctt. Even now, however, the etiology of true flacherie is not entirely clear. That the bacteria are secondary but active invaders to a rather benign virus has been postulated but not generally accepted. In any case, the sporeforming bacillus observed by Pasteur is associated with what is now called "true flacherie," and the streptococcus is associated with a similar disease known as "gattine." It is important to note that in conducting his researches on these diseases, Pasteur was well aware of the fact, now generally appreciated by insect pathologists, that the susceptibility of insects such as the silkworm is influenced by the conditions under which they live. Pasteur considered such factors as excessive heat and humidity, inadequate aeration, stormy weather, and poor food as inimical to the general physiological health of the insects, and capable of decreasing their resistance to infection.

Before leaving Pasteur, it behooves us to make special mention of his famous memoir, *Études sur la Maladie des Vers à Soie.* This is indeed a most stimulating and revealing document! As we read its pages and look at its excellent illustrations we can almost experience with Pasteur his initiation into the problem of infectious diseases. We can feel him becoming acquainted with the variability and unpredictability of animal life and behavior. The dawning of his realizations with respect to the laws of contamination, transmissibility, and epidemiology becomes apparent; and as we finish the treatise, we can understand his later enthusiasm for the principle of preventive medicine. This two-volume work, as well as some of Pasteur's other writings on the diseases of the silkworm, also reveals much of the human side of this man, the details of whose life are already so well known and chronicled. At times confident and boastful, at other times he was uncertain and humble. Even the preface of his memoir is interesting because of its concern with his personal feelings and attitudes. He begins by making a simulated apology for having undertaken the research for which he was so little prepared. When called to this task he had never so much as seen a silkworm! It is obvious, however, that he wrote these words with pride and and indulgence, knowing that he was presenting to the world not only a report of a successfully completed program of research, but a solution to a problem of great practical importance to his countrymen. Nevertheless, he did not refrain from reminding the reader of

his sacrifices, especially in so far as his work on the maladies of silkworms kept him from his pursuits in chemistry and fermentation, and of lamenting that more fame would probably have come to him had he stayed in fields of pure science. We cannot sympathize with him too much, however, when we realize that his work with silkworms greatly enhanced his insight into the phenomena of infectious disease generally. Indeed, were it not for his work on the diseases of the silkworm, who knows but that this French scientist might never have made his monumental discoveries on anthrax, rabies, septicemia, and other infectious diseases! Certainly these endeavors in medical science would have been delayed but for the fact that the lowly silkworm suffered from diseases that commanded the attention of Pasteur who, with Bassi, shares also the credit of initiating the real scientific development of insect pathology.

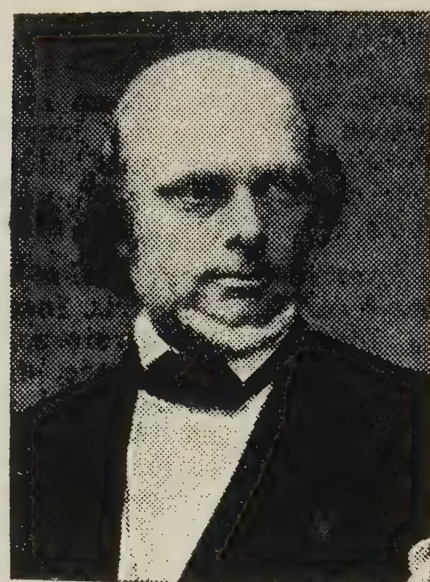
Other Developments

Other Protozoan Infections. Although biologists had been observing protozoa in insects prior to the time of Pasteur's studies on pebrine, it is generally acknowledged that greater attention was directed toward these entomophilic microorganisms as a result of the ravages of this disease among silkworms. Similar protozoa were revealed in other insect species, as well as in certain other animals. In 1882 Balbiani proposed the name Microsporidia as an order of the class Sporozoa in which to place the pebrine organism and related forms. A few years later Thélohan (1895) authored a notable monograph that included the Microsporidia, and Labbé (1899) presented a synopsis of genera and species of this group.

Other Sporozoa associated with insects were also being observed. Although some historians believe that Redi may have seen a gregarine in the seventeenth century, and Cavolini definitely described one from a crustacean in 1787, it was Dufour who, in 1826 and 1828, reported the presence of gregarines in insects and presented an authentic account of them as a group. Between 1851 and 1880 Leidy reported about 25 species of gregarines from arthropods. Other earlier observers who contributed significant information on species from insects included von Siebold (1839*b*), Lankester (1863), Bütschli (1882), and Léger (1892). Coccidia were observed infecting insects (*Gyrinus, Tipula,* and *Tineola* larvae) late in the nineteenth century (Schneider, 1885; Léger, 1897, and Pérez, 1899). (Smith and Kilbourne demonstrated the transmission of *Babesia bigemina* (Dennis), the cause of Texas cattle fever, by the tick *Boophilus annulatus* (Say) in 1893; and malaria parasites were seen in mosquitoes just before the turn of the century by Ross, in 1895 and 1897.)

Flagellates were first seen in insects about the middle of the nineteenth century. In most of these cases, however, the protozoan caused no appreciable harm to its arthropod host. Some of them, e.g., the flagellates of termites, are distinctly mutualistic. The first amoeba observed in an insect apparently was *Endamoeba blattae* (Bütschli), a commensal in the colon of the oriental cockroach, reported by Leidy in 1879. Amoebae truly pathogenic for insects were not discovered until after the turn of the century. The same must be said for the parasitic ciliates.

E. Cornalia



A. de Quatrefages

E. Verson

G. Bolle

Fig. 7. Four early investigators of diseases of the silkworm.
(Photos courtesy A. Schenk and C. Vago.)

Nematode Infections. It might be appropriate here to mention that nematode infections in insects have been known since the beginning of the nineteenth century, as we have already noted in our discussion of Kirby's (1826) account of diseases in insects. The scattered reports of entomophilic nematodes were brought together by von Siebold in a series of contributions between 1842 and 1858. Other prominent early contributors to our knowledge of insect nematodes include such men as Camerano, Diesing, Leidy, Leuckart, Linstow, and Lubbock.

Other Bacterial Infections. Knowledge of the bacterial diseases of insects might be said to have begun with Pasteur's (1870) study of flacherie of the silkworm. As we have explained in an earlier paragraph, he observed two kinds of bacteria associated with the diseased silkworms. Today we know these as *Streptococcus bombycis* and *Bacillus bombycis*, but their exact relationships to the diseases gattine and flacherie are still not entirely clear. During the remaining thirty years of the century, a considerable number of bacteria were reported from insects (see Steinhaus, 1946), but only rarely was their true pathogenic role demonstrated. It should be remembered that some of the so-called bacterial diseases of Lepidoptera, as described by certain of the early investigators, in actuality were virus diseases in which the bacteria isolated were secondary invaders or merely adventitious forms. This explains why so frequently the isolated bacterium never seemed to possess the virulence or capacity to cause epizootics that the natural disease exhibited. Nevertheless, some of the nineteenth-century studies of real and purported bacterial infections (e.g., those by Forbes, 1886; Krassilstschik, 1893; and Duggar, 1896) provided valuable factual information as well as stimulated interest in the diseases of insects from the viewpoint of controlling noxious species. In addition, they helped direct attention to the broader aspects of insect pathology and to the fact that insect pathogens could be found among any of the major groups of microbial life.

Undoubtedly, one of the most important bacterial pathogens of insects to be discovered during the nineteenth century was *Bacillus alvei* Cheshire and Cheyne, the cause of European foulbrood in the honey bee. (*Bacillus larvae* White, the cause of American foulbrood, was not discovered until 1904.) Cheshire and Cheyne described *B. alvei* in 1885, at a time when the various brood diseases were undifferentiated. It was not until well into 1900 that the confusion began to clear. Nevertheless, the seriousness of bee diseases had long been recognized. (We have already mentioned references to these maladies by Aristotle, Pliny, and Virgil.) In 1586, the German apiculturist Nickel Jacob not only described certain bee diseases, which he believed had their origins in putrefactions, but also suggested methods of combating them. In the years to follow, other European beekeepers became interested in and joined in the discussion of the various afflictions to which their bees were subject. Schirach (1771) was among the first to use the name "foulbrood" in reference to disease in the honey bee, and it was probably Dzierzon (1882) who first clearly recognized that there were at least two kinds of foulbrood. Numerous other investigations of bee diseases were conducted during the last half of the nineteenth century, and these have been ably reviewed by Phillips and White (1912).

Fig. 8. The first pictorial representation of polyhedral bodies in the tissues of an infected insect. There drawings were made from preparations of tissues from silkworms infected with the virus of silkworm jaundice. (From Maestri, 1856.)

While the early literature pertaining to the diseases of the honey bee is plentiful and interesting, the investigations it reported do not appear to have assumed as pivotal a role in the development of insect pathology during the nineteenth century as did the studies on the diseases of the silkworm. (During the twentieth century, on the other hand, the study of bee diseases assumed a much more significant part.) Moreover, the stimulus for the idea of using microorganisms to control harmful insects came largely from the silkworm disease studies rather than from investigations of the diseases of the honey bee. Nevertheless, a study of the early history of bee diseases enables the insect pathologist to learn of and note examples of the type of mistakes that it is possible to make in investigations of microbial diseases of insects.

Virus Infections. That insects are susceptible to infectious agents known as viruses was not clearly demonstrated until the second decade of the twentieth century (von Prowazek, 1907, 1912; Escherich and Miyajima, 1911; Glaser and Chapman, 1913; Acqua, 1919). While the most rapid and impressive advances in our knowledge of the virus diseases of insects have come during the past forty years, significant observations were made before this. Most of the important nineteenth-century developments were made while studying the polyhedrosis of the silkworm—a disease that has been designated by such names as "jaundice," "grasserie," "giallume," "Gelbsucht," and others. The Italian poet Vida may refer to this disease in his poem *De Bombicum,* written in 1527, and Maria Sibylla Merian mentions what is probably this affliction in a book on butterflies written in 1679. One of the earliest published descriptions of the disease itself is that by Nysten in 1808.

Early sericulturists confused jaundice with other diseases of the silkworm. Toward the end of the nineteenth century, however, it was generally recognized as a distinct entity, but the causative agent remained in doubt. Some workers (e.g., Hofmann, 1891; von Tubeuf, 1892; Krassilstschik, 1896) believed the disease to be caused by bacteria, but others recognized that the characteristic crystal-like bodies (polyhedra) regularly found in the tissues and body fluids of the diseased silkworms were somehow related to the cause of the disease. Among the first to make such observations and to associate these bodies with the disease were Cornalia (1856) and Maestri (1856)—exactly a century ago!

Cornalia (see fig. 7) described some of the symptomatological and pathological manifestations of silkworm jaundice and reported that the polyhedral bodies which he observed in the blood corpuscles originated from some kind of alteration of the blood. Maestri also observed the polyhedral bodies in the blood cells as well as in other tissues and called attention to their location in the nuclei of the cells (fig. 8). As far as the characteristic dissolution of the tissues was concerned, Maestri believed that the action of heat on the respiratory system of the silkworm brought about an alteration and a complete melting of the adipose tissue. According to Haberlandt (1871, 1872), who refers to the polyhedra as crystals, Verson was the first to recognize their crystalline nature, and Panebianco (1895) studied them from a crystallographic standpoint, likening them to rhombododecahedral crystals.

Bolle (1894, 1898) (see fig. 7), at first, also considered the polyhedra to be simply crystals; then he decided that they represented the sporulated form

of a protozoan parasite. He believed it to be a sporozoan that multiplied in a manner similar to that of coccidia, and his drawings depict a coccidianlike oöcyst filled with polyhedra, which he apparently believed were sporocysts. In any case, he correctly associated the polyhedra with the causative agent of the disease. He showed the polyhedron to be soluble in the alkaline juices of the silkworm gut, as well as in other alkaline and acid solutions, and observed that upon dissolution the polyhedron consists of a central granulated mass and a peripheral layer surrounded by a thin membrane. These observations are particularly noteworthy since today it is known that the virus particles are contained within the proteinaceous polyhedron which some believe to possess a membrane-like covering. The virus particles may be released from the polyhedron when the latter is treated with a dilute alkali.

Aside from the polyhedrosis of the silkworm, virus diseases of few other insects were being observed, and these usually under the impression that they were caused by bacteria. One of these, the polyhedrosis (*Wipfelkrankheit*) of the nun-moth caterpillar, first came to the serious attention of European entomologists in 1889 and 1892, and was destined to receive considerable attention in the years to follow. Forbes, in 1898 (*b*), described a polyhedrosis of the cosmopolitan armyworm, *Pseudaletia unipuncta* (Haw.). Although he could not ascertain the cause of the disease, he did describe the polyhedral bodies. Forbes also observed that the disease constituted an important natural check on populations of the insect.

Other Fungus Infections. In an earlier paragraph, I have referred to the early eighteenth-century observations of fungi associated with insects. The most spectacular of these fungi were species of *Cordyceps,* but examples of what appear to have been species of *Empusa* were also seen. After Bassi and others showed the white fungus associated with muscardine of the silkworm to be pathogenic in nature, other species of fungi were rapidly reported as parasitic on insects. Some of these reports will be referred to in the next section. Among those whose work and writings have had an important influence upon our basic understanding of entomogenous fungi in general are Robin (1847, 1853), Fresenius (1856, 1858), Gray (1858), the Tulasnes (1863–65), Brefeld (1870, 1877), Cohn (1870), Zopf (1890), Cooke (1892), Massee (1895), Giard (1896), and Thaxter (1888, 1896–1931). These last two workers deserve special mention.

Alfred Giard was an outstanding French mycologist who published forty papers on entomogenous fungi between the years of 1879 and 1896. Of particular importance were his contributions on certain Entomophthorales and on *Beauveria tenella* (Delacr.) (= *Beauveria densa* = *Isaria densa* (Link)). He studied fungus diseases of flies, locusts, various Lepidoptera, and the European May beetle, *Melolontha vulgaris* Linn. Most of his *Beauveria* researches were in connection with the latter insect. He studied the pathogenesis of fungus infections in insects and did much to clarify our understanding of the basic nature of these infections. He also offered (Giard, 1890, 1893) thoughtful advice concerning the use of entomogenous fungi in the control of harmful insects.

Roland Thaxter (1858–1932) was one of the leading American mycologists of his time, and certainly one of the world's outstanding students of ento-

mogenous fungi. He is best known for his two monographs: "The Entomophthoreae of the United States" (1888), and "Contributions Toward a Monograph of the Laboulbeniaceae" (5 vols., 1896–1931). Both of these contributions were of monumental importance. In addition, Thaxter studied, but published little, on such entomogenous groups as *Cordyceps, Isaria, Aschersonia,* Fungi Imperfecti, and others associated with insects. His life was one of dedication to scientific ideals as they could be applied to the groups of fungi that interested him. His contributions were largely taxonomic in character, and included presentations on development, host range, distribution, and other basic biological information. However, he apparently inspired such men as Speare and Rorer to be interested in the possible use of fungi in the economic control of insects.

Since an excellent account of Thaxter's life and works is readily available (Weston, 1933), it is unnecessary to repeat the details here. Suffice it to say that his published works were so thorough and meritorious that even today they are indispensable to students of Entomophthoraceae and Laboulbeniaceae. In the case of the latter, the first published record of one of these fungi was that by Rouget in 1850. One of the early leading contributors to the knowledge of the group was J. Peyritsch (1873). When Thaxter began his work on the group only a handful of species was known. When death disrupted his work in 1932, he had thoroughly described and magnificently illustrated literally hundreds of species. Unfortunately, his death prevented his finishing the sixth volume of his Laboulbeniaceae monograph in which was to be included a treatment of the general biological aspects of the group. Weston describes his monograph as ". . . one of the greatest single pieces of work of all time in mycology . . ." Because of their potential economic importance as insect pathogens, Thaxter's basic contributions to our knowledge of the Entomophthoraceae are especially valuable to present-day insect pathologists. Although the first published account of what was undoubtedly an *Empusa* infection appeared in 1776 (De Geer, 1776), and substantial contributions to the systematic knowledge of the group were made by Nowakowski (1883) and others, a real understanding of the group as a whole had to await Thaxter's monograph on the subject. This monograph represented his doctoral dissertation. Now that the increasing importance of these fungi as control agents is being realized, we can only hope that another candidate, possessing Thaxter's courage, devotion, and scholarship, will soon come forward to continue the work this American mycologist so nobly began.

THE EARLY DEVELOPMENT OF THE IDEA OF USING MICROORGANISMS TO CONTROL INSECTS

The serious observation and study of diseased insects had not progressed far before the idea of using disease to destroy noxious insects was conceived. Just exactly when, where, and by whom the idea originated are points on which we may never be absolutely certain, but we can avail ourselves of the story as it can be constructed from the available literature. In any event, the information so gleaned is exceedingly interesting and revealing. As is the case with many ideas, this one probably occurred to several men, at various

times, and in different parts of the world; and in all probability most of these conceptions went unrecorded. Nevertheless, it is fascinating to attempt to trace the development of the idea as best we can.

From the historical account we have already presented, we have seen how first came the observation that insects, such as the honey bee and the silkworm, are subject to disease. Then developed the realization that these diseases are of a contagious nature, and, later, that outbreaks of disease occur in insects in nature. As we proceed with our story it is well to keep this last point in mind since the awareness of it constituted a significant background to the more direct contributions to microbial control arising from the work on silkworm diseases. Early mycologists, for example, were aware that insects serve as natural hosts for numerous species of fungi (see Kirby and Spence, 1826; Gray, 1858; Cooke, 1875). Most of these early observers, however, were primarily interested in the nature and taxonomy of the fungus itself, and paid little attention to the fungus-insect relationship. Nevertheless, many insect hosts were recorded, and the idea that certain fungi could be considered as natural enemies of insects gradually became established. Furthermore, some of these biologists reported the occurrence of "pestilential epizootics" among insects in nature. Audouin (1839 *b*), for example, in 1838 observed the "disappearance" of *Galeruca calmariensis* Fab., a coleopterous pest of elms, as the result of an epizootic caused by the muscardine fungus (*Beauveria*). Hagen (1879) tells of an epizootic of "the common dung-fly" that occurred in 1867:

> Not only those, but many other insects died in the same locality and in the same manner; also other species of flies and gnats, the caterpillars of moths and of Phalaenids, and the common hairy caterpillar of a moth which is very nearly related to the famous hairy caterpillar of the Boston Common. Of some species the destruction was so complete that the next year they were very rare. . . . Similar observations have been made in other places in Europe and here. . . . In Entomological journals are reported fatal epizootics of leaf lice, of grasshoppers, of the cabbage butterfly and of the currant worm, both imported here only a few years ago, and both very obnoxious.

Other early reports of natural epizootics include those by Frauenfeld (1849), Köppen (1865), Bail (1867), Shimer (1867), Hagen (1869), Ratzeburg (1869), Cohn (1870), Brefeld (1877), and De Bary (1878).

The concept of using diseases to combat insects appears to have grown out of the observation that the maladies were infectious and contagious,[11] and that they could be transmitted from diseased to healthy individuals both in nature and experimentally. We have already seen how the transmissibility of the silkworm diseases was established. Naturally, the phenomenon as it applied to silkworms was soon carried over to diseases that occurred in other

[11] The earliest observers of muscardine in France, e.g., Boissier de Sauvages (1763) and Pomier (1763), did not consider muscardine to be contagious among silkworms. However, Nysten, in 1808, did consider it contagious under certain conditions. According to Robin (1853), Bonafous, in 1829, observed that muscardine is contagious. Not only silkworms but other larvae (*Phaloena verbasci* Linn.) placed in contact with silkworms dead of muscardine became infected in two or three days.

insects, as, indeed, Bassi (1835) himself accomplished experimentally in his efforts to prove the infectious nature of the white muscardine fungus, *Beauveria bassiana.* This pioneer not only artificially, by needle, infected numerous different species of insects (unnamed, but sometimes referred to as worms or caterpillars), but passed the fungus through long series of different insect species. Moreover, he declared that such transmissions could be accomplished whenever desired! Herein lies the germ of the thought that man can communicate agents of disease to susceptible insect pests at will.

Even more remarkable, however, is another contribution of Bassi's fertile and imaginative mind. In a footnote to a report of his on *negrone* (*flacherie*), Bassi (1836*a*) tells of finding that while the hemolymph extracted from normal larvae causes no harm when inoculated into another silkworm, if the fluid is first allowed to putrefy, the inoculated insect inevitably dies "in a state of *negrone.*" The same phenomenon occurs when putrefying substances such as milk and urine are used as inocula. Then he writes these important lines (freely translated):

> This fact considered, instead of using useless fumigations or medicated baths to kill the worms that destroy plants useful to us, one could try to spray their leaves with this water. The same worms nourishing themselves at any of the points touched by the poisoned liquid, even in the slightest portion, would unfailingly and quickly die. This bath or spray, far from being harmful to the tree, rather aids it, increasing its nourishment. Thus, by rotting a raw chicken egg, and after breaking it, throwing it into the water, one could prepare in this manner, if one wishes, several brentas [*brente*] of this exterminating liquid, with little expense.[12]

Thus, it would appear, on the basis of information available to the writer, that the credit and honor of having first suggested the possibility of employing the activities of microbial life to destroy insects harmful to man's interests belong to Agostino Bassi. To be sure, Bassi did not specify the use of microorganisms as such, but it is clear from the text accompanying the footnote referred to, that he conceived of the putrefying fluids as being of an infectious nature similar to the muscardine fungus on which he made his monumental studies.

Following the infectivity experiments of Bassi, others reported successful attempts to transmit artificially the infectious agent of muscardine to insects other than the silkworm. In 1836, Turpin was able to infect noctuids and other lepidopterous species with the fungus. A year later Audouin (1837 *b*, *c*) expressed the belief that muscardine is not peculiar to the silkworm, but appears among insects in general, and perhaps only among them. Later, in 1839 (*b*), he successfully transmitted the fungus by injection to several harmful insects including the gypsy moth. In the same year, Bonafous (1839) similarly transmitted the muscardine fungus to the larvae of several species of insects. Audouin (1839*a*) tells of a sericulturist who emptied contaminated

[12] Although the author has long been familiar with Bassi's writings and contributions to insect pathology, this particular passage (in Italian) from Bassi's 1836 work was kindly called to his attention by Dr. Enrico Masera of Stazione Bacologica Sperimentale, Padova.

silkworm-rearing trays out of a window onto trees whose leaves were being attacked by unidentified defoliating larvae. All of these insects were attacked by the fungus and died of muscardine four days later. Apparently, this fortuitous happenstance is the first reported instance of harmful insects in nature being destroyed by the artificial dissemination of a microbial pathogen. Robin (1853) and Metchnikoff (1879), apparently referring to this same instance, ascribe the reporting of it to Bonafous. The original brief note is designated as a communication to Audouin from Bonafous. Careful reading of the note, however, reveals that the reference to the experience of the unidentified sericulturist is made directly by Audouin as additional proof of his belief that muscardine can be spread by contact. This is also made clear in a subsequent note in which Audouin (1839*a*, p. 200) disclaims having recommended the procedure or having said it had been successfully employed. Perhaps he was attempting to protect himself from any evil consequences of the unnamed sericulturist's careless sanitation. In any case, he does not deny that the incident took place. From the standpoint of the development of microbial control, the kind of transmission referred to here was more significant than the type, for example, described by Lebert (1858) in which he was able to transmit the muscardine fungus from diseased to healthy insects when placed together in the same enclosure.[13]

Unfortunately, Bassi's visionary suggestion, as well as the Audouin report, apparently fell on barren soil for it was not until more than three decades later that we find the suggestion made again. This time it was made by the American entomologist J. L. LeConte (1874) (fig. 9) before the twenty-second meeting of the American Association for the Advancement of Science held in Portland, Maine, in August, 1873. The paper he read was titled "Hints for the Promotion of Economic Entomology," and in it he suggested a "new system of checks" to be employed against harmful insects. One of these checks was "the production of diseases." He proposed the communication of the muscardine fungus affecting silkworms to other lepidopterous larvae, saying: "I am extremely hopeful of the result of using this method. I have learned of an instance in which from the communication of the disease by some silkworms, the whole of the caterpillars in a nine-acre piece of woods were destroyed."[14] Toward the end of his paper he presents a number of recommendations among which is:

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[13] Some authors (e.g., Cooke, 1892) incorrectly state that Lebert's transmission experiments were done in trees, and imply that the work was done in 1826. Careful translation of the original account (1858, p. 178), however, reveals that the transmission experiments were done not in trees but "in einem Raume." The 1826 date refers not to the time of the transmission experiments but rather to the time when he first observed larvae of *Arctia villica* L. (=*Euprepia villica*) to suffer from muscardine.

An interesting type of transmission was postulated by Robinet (1843) who, as had others, observed small red "insects," called "Lentes" (lice), probably mites or other parasites, running about over the bodies of the silkworms, "stinging" them. He supposed that the spores of the fungus were carried by these arthropods and that infection occurred through the puncture wound.

[14] LeConte may refer here to an observation by Trouvelot (see Hagen, 1879*b*, *c*) in Massachusetts. Shortly after returning from Europe, in 1867, with silk-producing moths which apparently were infected with the muscardine fungus, he observed the disease in a population of *Polyphemus* moths (and other species) he was rearing for silk in 12 acres of shrub land.

Careful study of epidemic diseases of insects, especially those of a fungoid nature: and experiments on the most effective means of introducing and communicating such diseases at pleasure.

Fig. 9. John Lawrence LeConte (1825–1883), American entomologist and one of the earliest to suggest the use of microorganisms in the control of insect pests. (Photo courtesy Professor E. O. Essig.)

To the best of our knowledge, LeConte's recommendation represents the first clear-cut suggestion advocating the use of disease as a means of insect control to appear in the English language. To be sure, he undoubtedly derived the idea from the observations of others on the diseases of the silkworm, but LeConte's proposal was definite yet broad in its concept, and clearly envisioned the practical possibilities involved (see also Lesley, 1880).

It is highly interesting and significant that another early definite sugges-

tion that microorganisms might be used to combat insects came from Louis Pasteur—undoubtedly as a result of, or an afterthought from, his classical work on the diseases of the silkworm. He published this suggestion in 1874 at a time when the grape phylloxera was threatening grape production in France. He had suggested the use of "les corpuscules de la pébrine" against this pest, apparently assuming that it would be susceptible to the protozoan. Then he recommended that a search be made for a fungus that was capable of destroying the insect, and that such a fungus be introduced into vineyard populations of phylloxera. Although he never tried to establish the practical usefulness of fungi against phylloxera or other insects,[15] Pasteur apparently thought the idea had possibilities for (as told by Dubos, 1950) in 1882, almost ten years later, he dictated the following laboratory note to his assistant Adrien Loir:

> To find a substance which could destroy phylloxera either at the egg, worm, or insect stage appears to me extremely difficult if not impossible to achieve. One should look in the following direction.
>
> The insect which causes phylloxera must have some contagious disease of its own and it should not be impossible to isolate the causative microorganism of this disease. One should next study the techniques of cultivation of this microorganism, to produce artificial foci of infection in countries affected by phylloxera.

In 1884, however, Balbiani, also expressing the idea of using the pebrine organism to control the phylloxera, pointed out some of the problems that

[15] Pasteur was later, in 1887 and 1888, to advocate and test the use of a pathogenic bacterium (the fowl-cholera bacillus, *Pasteurella multocida* (L. & N.)) to destroy rabbits. On the Pommery estate near Rheims, where rabbits had become a nuisance in a wine cellar, his assistant, Loir, conducted an anti-rabbit campaign Pasteur had outlined. Within three or four days after placing the bacteria on cut alfalfa around the burrow openings, thirty-five rabbit cadavers were found, and no living rabbits were in evidence. Later additional dead rabbits were found in the burrows. These promising results induced Pasteur to send Loir to Australia to organize an antirabbit campaign there in response to that government's plea for an effective method of exterminating the animals. This project was never carried out, however, because the necessary authorization was never granted by the Australian Department of Agriculture. In recent years in Australia, and in Europe, the mosquito-transmitted virus of infectious myxomatosis has been used to bring about epizootics in rabbits. In some areas marked declines in the rabbit populations were effected (see Fenner and Day, 1953).

Other efforts have been made to use disease organisms to destroy animal and plant pests. Best known perhaps are those attempts to control rats and mice with *Salmonella typhimurium* (Loeffler) (e.g., see Danysz, 1893, 1900; Rosenau, 1901). The idea of destroying noxious weeds originated at about the same time as did similar ideas of destroying insects, as evidenced by Peck's suggestions along this line in 1876.

It has been only during the past quarter century that serious consideration has been given to the use of microbial pathogens in warfare between men. Fortunately, this debasement of science is not a product of, or in any way associated with, the development of applied insect pathology. Of considerable reassurance for students of insect pathology and those concerned with the microbial control of insects is the fact that the products of their labors cannot in any way contribute to biological warfare. Indeed, the contributions of insect pathology to agriculture, medicine, and biology generally are such that only humble pride, not apprehension, need accompany the work of those engaged in the study of the diseases of insects.

would be involved, such as how to infect the sucking insect (if, indeed, this were possible), and how to distribute the pathogen in the soil of the vineyard. He then states that the idea appears to have been abandoned.

Scientific observations and reports are frequently important, not because of the factual information they may contribute, but rather because of the influence they may have on the thinking of others. Such a characterization may be applied to certain transmission experiments and reports, factually erroneous, conducted by Carl Adolph Emmo Theodor Bail of Danzig. His experiments were significant primarily because of the impression they made on H. A. Hagen who later, in the United States, advocated the use of fungi to destroy insects. At European meetings of an association of naturalists, in 1861, Bail delivered lectures during which he exhibited a mold grown on a mash that had been sown with the "fungus of the house-fly," as well as a keg of beer brewed from such mash, and a cake baked with what Bail considered to be the yeast form of this fungus.[16] This Prussian worker maintained that the fungus was capable of killing such insects as flies, mosquitoes, and caterpillars brought in contact with the inoculated mash. There is no record, however, of his having advocated the practical use of fungi in the control of harmful insects.

Impressed by the work and writings of Bail, Hagen (fig. 10), in 1879 (*a*, *b*, *c*), published a paper[17] in which he expressed "the conviction that a remedy for insect pests, offering several prominent advantages, could be found in the easy application of the yeast fungus." Also, ". . . I believe I should be justified in proposing to make a trial of it against insect calamities." He then proceeds to make rather specific suggestions, as follows:

> Beer mash or diluted yeast should be applied either with a syringe or with a sprinkler; and the fact that infested insects poison others with which they come in contact will be a great help. Of course it will be impossible to destroy all insects, but a certain limit of calamities could be attained, and I think that is all that could reasonably be expected. In greenhouses the result would probably justify very well a trial, and on current worms and potato bugs the experiment would not be a

[16] On the basis of present-day knowledge, it is obvious that, from a systematic viewpoint, Bail's mycology was considerably in error, as was pointed out by some of the botanists of that day. He believed that four different species were but different forms of the same fungus. Thus the house-fly fungus (*Empusa*) appeared as a "common mold" on vegetable matter, as a yeast under conditions conducive to fermentation such as on a mash, and as a water mold (*Saprolegnia*) when grown in water. Presumably, either the form occurring on house flies or the yeast form was capable of killing insects. In light of our understanding of these forms today, four distinct species are represented and of them only the house-fly fungus (*Empusa*) is pathogenic to insects; the yeast (*Saccharomyces*) referred to almost certainly was, in itself, quite harmless to insects.

Of interest is the fact that the so-called house-fly fungus was not always considered a boon as the result of its activities. Hagen (1879*b*, *c*) refers to it as "the vexation of every housekeeper. The dead flies stick in the fall firmly to the windows, or anywhere else, and are covered by a white mould not easy to be removed."

[17] This paper was first published in *The Boston Evening Transcript* on April 11, 1879, and then appeared in the May, 1879, number of *Le Naturaliste Canadien*, Vol. 11, pp. 150–155, and then in the June, 1879, issue of *The Canadian Entomologist*, Vol. 11, pp.110–114. Following this, Hagen had the paper reprinted, with some revisions and additions, as a bulletin by the Cambridge University Press, December, 1879.

difficult one, as the larvae of both insects live upon the leaves, which can easily be sprinkled. But it seems to me more important to make the trial with the Colorado grasshopper. I should recommend to infest the newly-hatched brood, which live always together in great numbers, and I should recommend also to bring the poison, if possible, in contact with the eggs in the egg-holes, to arrive at the same results, which were

Fig. 10. Hermann August Hagen (1817–1893), German-American entomologist who was one of the early scholars who advocated the use of microorganisms to control noxious insects. (Photo courtesy Professor E. O. Essig.)

so fatal to Mr. Trouvelot's silk-raising. After all, the remedy proposed is very cheap, is everywhere to be had or easily to be prepared, has the great advantage of not being obnoxious to man or domestic animals, and if successful would be really a benefit to mankind. Nevertheless, I should not be astonished at all if the first trial with this remedy would not be very successful, even a failure. The quantity to be applied and the manner of the application can only be known by experiment, but I am sure that it will not be difficult to find out the right method.

Thus Hagen joined Bassi, LeConte, and Pasteur in being among the first to make original and concrete proposals to attempt the use of microorganisms to destroy and control noxious insects. Except for Bassi's (1836) suggestion, these proposals were made during a six-year period, from 1873 to 1879, during which none of these men actually conducted experiments to test their ideas. But experimentation, with an eye to the practical applications of the method, was not long to follow. In fact, cursory and inquiring experiments were conducted during the same year, 1879, in which Hagen made his proposals.

Acting upon the suggestions made in Hagen's paper, J. H. Comstock, C. V. Riley, and J. H. Burns conducted separate experiments, in breeding cages, using suspensions of commercial yeast in efforts to destroy "caterpillars," "cotton worms," and "potato bugs" (see Hagen, 1879*c*; also Comstock, 1879). Comstock and Riley reported negative results, as did Prentiss (1880), Willet and Cook, and others, in separate series of experiments. Burns wrote Hagen that insects sprinkled with the yeast solution died from eight to eleven days following treatment, whereas insects not so sprinkled showed little mortality. In the wing blood of some of the supposedly diseased specimens sent to him, Hagen found "spores of the yeast fungus in quantity." On the basis of these experimental results, Hagen came to the conclusion that "the application of yeast on insects produces in them a fungus which becomes fatal to the insects." Later, Hagen (1880*b*, 1882*a*, *b*) reported additional successful results after the yeast had been used against aphids and "currant worms." However, since, in general, varied results were being obtained by those who tried the yeast, Hagen felt obliged to postulate that "a *certain stage* of the yeast solution is needed to make it effective." From 1882 on, very little was written concerning the use of commercial yeast to kill insects. This is understandable since certainly it cannot be considered a pathogen of insects. Unless the solutions tested were contaminated with pathogenic forms, it must be assumed that the mortality attributed to the yeast was coincidental or, at least, unrelated to this microorganism. For our purposes here, however, it matters little that the results of the experiments with yeast were misjudged by Hagen, and—as referred to by De Bary—amounted to an "item in the history of error." The significant thing is that he, along with LeConte and Pasteur, saw and advocated the potentialities of microbial control within the limits of microbiological knowledge of that time.

About the same time that the American workers were concerned with the use of yeast as an insecticide, a notable development in the idea of applied insect pathology was also taking place in eastern Europe. Here a young zoologist, destined to become famous for his studies on phagocytic immunity and to receive the Nobel Prize, turned his attention to a serious agricultural problem. Elie Metchnikoff (1845–1916) (fig. 11) found himself concerned over the great amount of destruction that was caused to cereal crops in Russia by the "grain beetle" or wheat cockchafer, *Anisoplia austriaca* Hbst. He was impressed by the rise and fall of the populations of this pest in different years, and believed that such oscillations could be caused by outbreaks of disease among the insects.

Beginning in the autumn of 1878, in the region of Odessa, Metchnikoff

found three distinct diseases in *A. austriaca*; one of the maladies was caused by one or several kinds of bacteria or "vibrions," another by a nematode, and the third by a fungus which he named *Entomophthora anisopliae* (later *Isaria destructor*) and which is now known as *Metarrhizium anisopliae* (Metch.) Sorokin. This fungus, since found infecting numerous insect species, causes a disease which Metchnikoff called "green muscardine," and which is characterized by the dark green color of its conidia, or spores. He studied the fungus from a mycological viewpoint as well as from a pathological one.

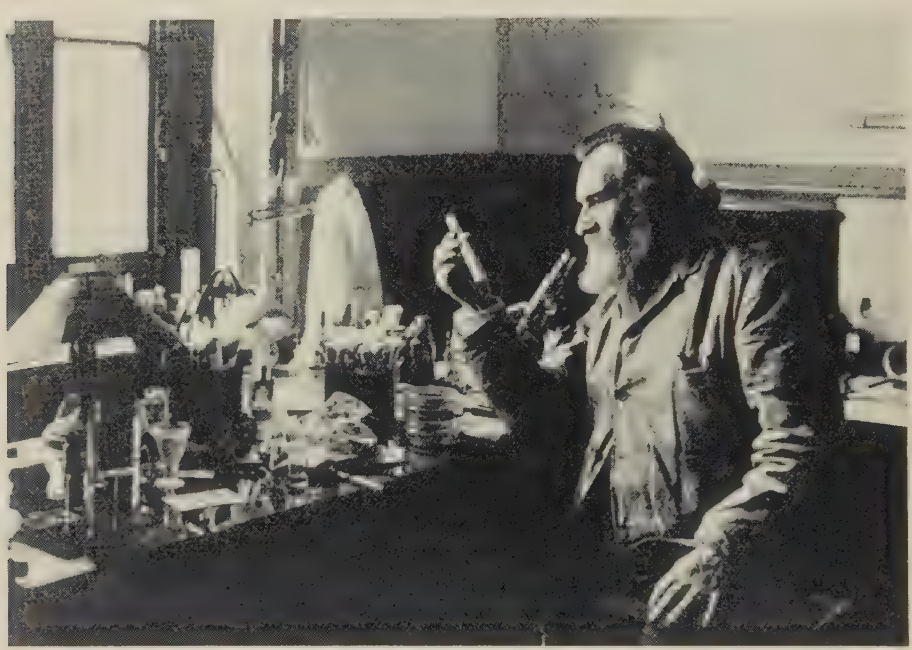

Fig. 11. Elie Metchnikoff (1845–1916), Russian zoologist and among the first to initiate the experimental approach to the use of microorganisms in the control of harmful insects. (From "Life of Elie Metchnikoff" by Olga Metchnikoff (1921), Constable and Company, Ltd., London.)

Especially noteworthy is the fact that Metchnikoff (1879) appreciated the significance of natural epizootics in reducing insect populations, that he envisioned the practical use by man of disease agents, especially fungi, in the control of insects, and that he tested this possibility experimentally. He suggested scattering about the infested fields the bodies of larvae dead of green muscardine, the soil in which diseased larvae had been found, or the free spores of the fungus itself. Moreover, he recommended that for greater success in the conduct of the operation, nurseries should be established at various locations for the purpose of producing the pathogenic fungus. Metchnikoff believed that natural epizootics, in themselves, could not be depended upon to control a destructive insect, but that with man's participation effective suppression of the pest might be attained; man may not only find means of

effectively disseminating the pathogens, but may also find means of intensifying their virulence and activity.[18]

In his 1879 report, Metchnikoff tells of experiments in which he found that healthy *Anisopliae* larvae placed in earth already containing diseased larvae would acquire the fatal disease. Furthermore, and obviously with an eye to the method's practical application, he was able to bring about the disease by mixing the fungus spores themselves with the soil into which the larvae were to be placed.

From the standpoint of chronology, it might be pointed out that while Metchnikoff's advocacy of microbial control methods followed by several years similar suggestions by LeConte and Pasteur, and by students of forest insects in Germany, his initial experimental work apparently preceded by several months that of the American workers who tested Hagen's proposals. It is worth noting, however, that Metchnikoff (1880) himself refers to "similar results" obtained by De Bary in experiments with the fungus *Isaria farinosa* (Dicks.).

During the year 1879, Metchnikoff (1880) found infected *Anisopliae* larvae in other regions of southern Russia; he also found the disease affecting the sugar-beet curculio, *Cleonus punctiventris* Germ. In the case of the latter insect he estimated that 40 per cent of the natural population was destroyed by the fungus. Experimental tests confirmed the susceptibility of the weevil. Upon Metchnikoff's suggestion to use the fungus to control the insects, entomological commissions in Kharkov and Odessa were assigned to investigate the matter further. In the meantime, Metchnikoff, after seeking methods to propagate the fungus artificially, discovered (upon the suggestion of the chemist A. Werigo) that spores could be produced on sterilized beer mash. (He appears to have been the first to realize the importance of the mass production of entomogenous fungi or their spores by "artificial" means.)

At this point, apparently, Metchnikoff's attentions were directed toward other problems. Just what took place is not clear. However, Madame Olga Metchnikoff in her "Life of Elie Metchnikoff" (1921) records the following cryptic paragraph:

> At first he confined himself to laboratory experiments; then a great landowner, Count Bobrinsky, placed experimental fields at his disposal. As the acquired results were very encouraging, Metchnikoff, forced to leave the neighborhood, left a young entomologist in charge of the application of his method. So far as he himself was concerned, this study proved the starting-point of his researches on infectious diseases.

We know of no scientific record of the field experiments to which Madame Metchnikoff alludes. From what is known, however, it appears clear that Metchnikoff's work and ideas (in all probability influenced by those of con-

[18] Like Pasteur, Metchnikoff (1879) advocated the use of microorganisms to control the grape phylloxera. He believed that such control was possible because of Leydig's (1854, 1863) report of "pébrine corpuscules" in *Coccus hesperidum* Linn., which he considered to be a "close relative" of phylloxera. It now appears that what Leydig and others had mistaken for microsporidian spores were in fact the yeastlike symbiotes characteristically present in coccids.

temporaries such as Pasteur and De Bary) gave genuine impetus to the idea of microbial control, and accomplished and inspired the first significantly practical results to be attained by such means. We see evidence of this a few years later in the work of I. Krassilstschik; in the meantime, Metchnikoff's observations, as had those of Hagen, caught the speculative attention of a few entomologists and biologists (e.g., see Lankester, 1880).

Isaak Krassilstschik, following the lead of Metchnikoff, in 1884 organized a small production plant in Smela. After about four months of operation, 55 kilograms of *Metarrhizium* spores were produced (Krassilstschik, 1888). These spores, mixed with fine sand, were scattered about in certain field plots in the vicinity of Kieff. After from 10 to 15 days, from 55 to 80 per cent of the *Cleonus* larvae in the plots were dead of green muscardine. In spite of this encouraging beginning, however, the work was not continued, apparently (according to a letter from Krassilstschik to Giard and quoted by Paillot, 1933) because of a rather sudden cessation in the production of sugar beets that made it unnecessary to control *Cleonus,* although some Russian authors (Rubtsov, 1948; Pavlovsky, 1952) ascribe the abatement of the work to the failure of the method to give consistently successful results which, in turn, was caused by a lack of understanding of variations in virulence and the basic epizootiological factors involved.

As a result of the stimulus of Metchnikoff's observations and recommendations, the next two decades were to see the gradual acceleration of interest in the possible use of fungi to control insect pests. In Europe, Brongniart (1888) advocated the scattering of pulverized fungus-infected insects as well as spores of entomophthoraceous fungi among the larvae of flies and other agriculturally important insects as a means of inexpensive control. Similar recommendations were made by Künckel de Herculais and Langlois (1891) with regard to certain grasshoppers. In 1892 the physiologist Franz Tangl (1893) attempted to use the white-muscardine fungus, *Beauveria bassiana* (Bals.), against caterpillars of the nun moth, *Lymantria monacha* Linn. His laboratory experiments were successful, but in nature the trees sprayed with spore suspensions were not protected against the insect, possibly because conditions of adequate moisture did not prevail at the time. Similar negative results were obtained by von Tubeuf, about this same time, using *Cordyceps militaris* (Link). Among other nineteenth-century European workers who experimented with and wrote on microbial control methods for controlling insects were Giard (1890, 1892, 1893), Dufour (1891), Príllieux and Delacroix (1891), Danysz (1893), Sauvageau and Perraud (1893), and Trabut (1898*a, b;* 1899). Giard's work with and attempts to use *Beauveria tenella* (Delacr.) (=*B. densa* (Link)) against *Melolantha* were particularly interesting.

Several methods of producing the spores in quantities for field distribution were tried by Giard and others (see also Kellogg, 1894). The results of the field trials varied—sometimes the results were excellent, and at other times discouraging. The outcome appeared to depend largely on environmental conditions, of which an adequacy of moisture began to emerge as of primary importance. Giard himself concluded that the use of the fungus gave favorable and encouraging results but only under the appropriate con-

ditions. This worker also offered sage advice against unwarranted generalizations on the use of fungi against noxious insects. Especially did he protest dangerous popularizations of microbial methods; he believed strongly that the approach should be one that is serious, careful, and completely scientific —a viewpoint that can still be heartily endorsed. He was not without hope of eventual practical achievements, however, if man but diligently and carefully worked to reveal the secrets of nature involved: "Nous faire enfin, des alliés de ces terribles cryptogames que nous avons si malheureusement appris à connaître comme des adversaires redoutables, n'est-ce pas une oeuvre digne de tenter bien des bonnes volontés, de mettre en mouvement bien des intelligences?" Also worth repeating is the assertion by Dufour who pointed out that the difficulty in using fungi to destroy insects is not in finding the necessary entomogenous fungi (of which hundreds of species abound and are relatively well known) but in knowing how properly to use these fungi to cause epizootics at will.

Meanwhile, in the United States, there was beginning to unfold what was to become one of the best-publicized, and in some respects least-understood, chapters in applied insect pathology. Indeed, in all probability the final pages of this chapter are still to be written. And although man has not succeeded in mastering the use of the fungus concerned, so many basic lessons were learned as a result of the project that the time, money, and effort involved were eminently worth while. We are referring to the attempts made in midwestern United States to control the chinch bug, *Blissus leucopterus* (Say), a serious pest of cereal crops, by means of *Beauveria globulifera* (Speg.) (=*Sporotrichum globuliferum* Speg.). Most of the salient features of this story have been told elsewhere, so it is our intention here merely to refer briefly to these earlier accounts.

Although a white fungus on chinch bugs was observed in 1865, by Shimer (1867), in Illinois, the first certain record of *Beauveria globulifera* on *Blissus leucopterus* was that by Forbes (1890) in 1887, in Clinton County, Illinois. Shortly thereafter it was reported from Minnesota, Iowa, Ohio, and Kansas. (The chinch bug was first noticed in the United States, in North Carolina, in 1783—more than a hundred years earlier.) The first attempt to bring about an outbreak of the disease by artificial dissemination was that by Lugger, in 1888, who scattered diseased bugs about the fields in several localities in Minnesota. Although the experiment appeared successful, Lugger suspected that, since the disease spread so rapidly, the spores of the fungus were already present in the test fields and that he had only reintroduced them.

In 1888 F. H. Snow (1890, 1891, 1894, 1895, 1896), in Kansas, began his work on the chinch bug fungus. The Kansas state legislature established an "experimental station" at the University of Kansas to propagate the fungus and to distribute it free of charge. It was placed under Snow's direction. Almost 50,000 packages of the fungus were distributed by this station, but the true value of the program was never ascertained with certainty. The reports of observers in 1891 and 1892 were very favorable, whereas those made during succeeding years were less favorable. Distribution programs were carried out in states other than Kansas, but in each case the work was even-

tually dropped. Lugger in Minnesota tried the method again in 1895 but gave it up by 1902. It was similarly abandoned in Illinois, Nebraska, Missouri, Ohio, and Oklahoma. Although natural outbreaks of the disease were frequently very effective in reducing large populations of the insect, the artificial distribution of the fungus did not appear to affect materially the incidence of the disease or the effectiveness of the control. Possible reasons for this were analyzed in a report by Billings and Glenn (1911) who made a comprehensive study of the disease and the distribution programs, and concluded that, because of its wide natural presence, the artificial distribution of the fungus was of little or no value. In spite of the abandonment of this program, it is to the credit of F. H. Snow that he did much to awaken entomologists, as well as farmers, to the potentialities of this type of biological control. We might also remember that Snow (1891) recommended that his State of Kansas should have "a bacteriological laboratory" in which the relation of bacteria to insects, as well as to human beings and domestic animals, might be studied.

Similar credit must be extended to S. A. Forbes who apparently nurtured a deep interest in insect pathology. His concern with the diseases of insects antedated his studies on the chinch bug fungus, and included bacterial diseases (and unknowingly some virus infections), as well as those caused by fungi (Forbes, 1882, 1883, 1886, 1888, 1895*a*, *b*, *c*, and 1898*a*, *b*).[19] His efforts were "directed especially to the point of artificially propagating [the diseases] for the destruction of injurious insect species." His observations on the chinch bug fungus were, in general, careful and discerning, and at several points he anticipated the findings and conclusions of Billings and Glenn. Like Snow, he ably administered the distribution of fungi among growers in a manner designed to obtain the type of effective cooperation required in such endeavors. Thus, as the century approached its close, we find at least two leading American entomologists among those who were willing to investigate the possible use by man of microorganisms that in nature could be so effective in reducing populations of harmful insects. And, in the writings of these men can be detected the influence of the early work on the diseases of the silkworm. We can see again how the idea of using microorganisms to destroy economically important insects had its roots in the silkworm studies. There is an account (see Riley, 1883) of a paper presented by Forbes before an entomological club in which he suggested the possibility of using contagious diseases of caterpillars for economic purposes. In this paper he refers to Pasteur's 1869 experiments of the contagious nature of flacherie of the silkworm, indicating that his (Forbes') ideas for microbial control were stimulated by this earlier work on the diseases of a beneficial insect.

Thus we come to the close of the nineteenth century with the use of microorganisms just emerging as a potential method of controlling insects. The role of microbial pathogens in the natural control of many species of insects

[19] For an interesting and valuable list of annotated references to the American literature of insect pathology between the years of 1824 to 1894, the reader is referred to one of Forbes' (1895*c*) excellent reports as State Entomologist of Illinois. From this list it is apparent that the American contribution to the beginnings of insect pathology was substantial. A similar, although less complete, list of references also appeared a few years earlier in *Psyche* (Forbes, 1888).

was recognized; the secrets as to how nature accomplished this, however, were inadequately known—as, indeed, they are today although to a somewhat lesser extent. As the century ended, virtually all of the thinking with regard to microbial control methods was concentrated on the possible use of fungi. Although understandable, this was to some extent unfortunate inasmuch as the successes and failures of entomogenous fungi colored the approach that was to be made with other infectious agents. Nevertheless, 1900 probably represents the approximate date of the high-level mark as far as the hopes of early workers were concerned, and it ushered in a period of intense activity and enthusiasm that was to persist until the 1930's when skepticism and discouragement were general. Around 1940, however, as the potentialities of milky disease in controlling the Japanese beetle became apparent, a new phase in the history of insect pathology began. Fresh approaches were being made especially in the United States and in Canada. There developed a deeper appreciation of the necessity of accomplishing a greater amount of basic or fundamental research before the effects of disease on insect populations could be thoroughly understood. The lessons taught by past attempts to utilize microbial control methods were being absorbed. New laboratories with staffs specially trained in phases of insect pathology were being established in various parts of the world. No longer need insect pathology be a stepchild of other disciplines, but a distinct and legitimate branch of entomology. Although still in its youth, it is fast coming of age, and with it the idea of microbial control is developing. This twentieth-century progress is another story, but one well worth telling. When it is told we hope that the account we have presented here will testify to the heritage of insect pathology, and to the greatness of the men who were its pioneers.

CONCLUDING STATEMENT

A recapitulation of the historical details recounted in this paper is probably unnecessary since the article in itself is in reality but a summarization of the history of insect pathology from the time of Aristotle until 1900. We can only hope that we have succeeded in our principal objective which was to trace the birth and growth of the idea of microbial control through the earliest years of its development. Accordingly, instead of a summary at this point, we should like to conclude with a few comments addressed to the relevance and significance of the story we have tried to depict. A chronology of some of the principal events and developments in the early history of insect pathology is presented at the end of these concluding remarks.

In the first place, it is clear that the idea of using microorganisms to destroy noxious insects had its origins largely in studies of the diseases of the silkworm. Beginning with Bassi, in 1835, and subsequently aided by such men as LeConte, Pasteur, and Hagen, the concept of microbial control had matured enough so that by 1879, Metchnikoff was able to initiate experiments testing its feasibility. Significantly, even considering the rapid developments of recent years, most of the basic scientific principles of our knowledge of infectious disease in insects have come to us from studies of the diseases of the silkworm. (These studies also contributed greatly to the early understanding of infectious diseases in man.) It is well that we give this magnificent

insect the added distinction of having, through its maladies, inspired early experimenters and interested biologists to conceive of a possible new method of controlling insects harmful to man's welfare.

It is important, too, to note the principal chronological steps in the evolution of the idea of microbial control; i.e., the realization: (1) that insects, such as the silkworm, were subject to disease; (2) that the diseases were of a contagious nature; (3) that natural outbreaks of disease occurred; (4) that insects served as natural hosts for certain species of fungi; (5) that the agents of the diseases could be transmitted from diseased to healthy individuals; (6) that the diseases were caused by living agents (microorganisms) that could be dispersed; and (7) that these microorganisms could be grown in numbers and quantities large enough for field distribution. To be sure, insect pathologists have since learned that a great deal more than the mere distribution of infectious agents is involved in the successful use of microbial control methods. Nevertheless, the development of the idea of microbial control to the point where the distribution of pathogenic microorganisms was seen as a possible means of destroying noxious insects was, indeed, a mighty stride of the first importance.

Among the benefits to be gained from a careful perusal of the early history of insect pathology are an appreciation and knowledge of the types of mistakes and errors to which the disciplines involved are subject. The serious student can truly learn from the mistakes of his learned predecessors. He learns also that even such a giant as Louis Pasteur, who for two years refused to admit the infectious nature of the pebrine pathogen, may be fallible in details although eminently right in basic principles. The serious student sees how the work of one man is interwoven with or is built upon and influenced by that of another. And, if he is the least bit generous with his admiration, he will appreciate the difficulties, from both the material and morale standpoints, that faced the fathers of his science. Unless he is entirely calloused to such emotion, the student of historical science cannot help but be genuinely inspired and excited by the thoughts, deeds, and achievements of those who first studied the diseases affecting insects. Furthermore, he will experience considerable satisfaction in the knowledge that insect pathology has contributed significantly to agriculture, medicine, biology, and even philosophy.

Since the progress of human endeavor depends on man's ability to control the forces of nature, the emergence and development of the idea of microbial control is important for its contribution to this end. During the past half century the ability to harness the activities of microscopic organisms in the service of mankind has been one of the greatest of all scientific achievements. Notable examples are the use of microorganisms in the manufacture of food, clothing, and shelter, and in the production of life-saving antibiotics. In much the same spirit there is reason to believe that the use of microorganisms may have a significant place among the efforts of man to protect himself from the ravages of insects—his number one competitor on earth. If such should prove to be a practical reality, the revelations and developments in insect pathology during the eighteenth and nineteenth centuries will take on even greater significance and splendor than they do now—and their present magnificence is eminently worth our admiration, study, and understanding.

CHRONOLOGY OF SOME OF THE PRINCIPAL EVENTS AND DEVELOPMENTS IN THE EARLY HISTORY OF INSECT PATHOLOGY

335–322 B.C.—Aristotle described certain diseases of the honey bee in his *Historia Animalium.*

37–29 B.C.—Virgil mentions the diseases of bees in his *Georgics.*

77 A.D.—Pliny refers to afflictions of the honey bee in his *Libros Naturalis Historiae* [*Historia Naturalis*].

1527—Marcus Hieronymus Vida published a poem on the silkworm, and includes a passage on the diseases of the insect.

1679—Diseases of the silkworm referred to in a book on butterflies written by Maria Sibylla Merian.

1726—First published record of an identifiable fungus (a *Cordyceps*) parasitizing an insect. This was a paper by R.-A. de Reaumur who relayed a report on the "Chinese plant worm" by Parennin.

1760—Saprophytic fungi growing on the bodies of insects immersed in water are first reported by Ledermüller.

1771—Schirach first used the term "foulbrood" in reference to disease in the honey bee.

1776—De Geer published what is probably the first description of what we now know as an *Empusa* infection in flies.

1805—Latreille referred to a disease of domestic flies that apparently was caused by an *Empusa* fungus.

1808—Publication of a treatise ("Recherches sur les Maladies des Vers à Soie") by P. H. Nysten on the diseases of the silkworm. First comprehensive scientific treatment of the diseases of this insect.

1821—Foscarini showed experimentally that muscardine of the silkworm was infectious.

1826—The appearance of a chapter titled "Diseases of Insects" by William Kirby in Kirby's and Spence's "An Introduction to Entomology." A remarkable presentation for that time.

1828—Dufour reports the presence of gregarines in insects.

1834—Agostino Bassi, for the first time, showed experimentally that a microorganism (the fungus *Beauveria bassiana*) was the cause of an infectious disease in an animal (the silkworm).

1835—Bassi showed that insects other than the silkworm were susceptible to the muscardine fungus.

1836—Bassi was the first to suggest that microbial life (putrefying substances) be used to destroy harmful insects.

1836—Turpin successfully infected noctuids and other Lepidoptera with the muscardine fungus.

1839—V. Audouin transmitted muscardine fungus to several species of harmful insects. He also reported that an unidentified sericulturist emptied fungus-contaminated silkworm-rearing trays out of a window onto trees infested with harmful insects. These insects apparently thus contracted the disease.

1847—C. Robin published an important review of the entomogenous fungi.

1856—Important works that included a coverage of the diseases of the silkworm were published independently by A. Maestri and by E. Cornalia. Polyhedral bodies characteristic of the virus-caused jaundice of the silkworm were first described by these men.

1858—G. R. Gray published a comprehensive review of *Cordyceps.*

1861—C. A. E. T. Bail claimed yeast fungus to be same as "house-fly fungus," and maintained it to be capable of killing insects such as flies, mosquitoes, and caterpillars. Although this work was mycologically erroneous, it inspired H. A. Hagen to become interested in advocating microbial control methods.

1865–1870—Louis Pasteur conducted his experiments on the diseases of the silkworm known as "pébrine" and "flacherie."

1867—A. Béchamp correctly identified the pébrine corpuscles as spores of a parasitic microorganism (the microsporidian *Nosema bombycis* Naegeli).

1870—Pasteur's monumental work *Études sur la Maladie des Vers à Soie* was published.

1873—J. L. LeConte first definitely recommended the study of diseases of insects to determine the most effective means of using them against noxious species.

1874—Pasteur suggested the use of microorganisms to combat the grape phylloxera in France.

1879—H. A. Hagen advocated use of a yeast fungus to destroy noxious insects.

1879—Elie Metchnikoff published an important paper in which he reported the natural infection of the wheat cockchafer, *Anisoplia austriaca* Hbst., by the green-muscardine fungus, *Metarrhizium anisopliae* (Metch.), expressed appreciation of the significance of natural epizootics in reducing insect populations, envisioned the practical use by man of disease agents in the control of insects, and reported preliminary experimental tests of the method.

1879—Alfred Giard began his work on various entomogenous fungi. He published a series of papers on this subject between the years of 1879 and 1896. Recognized potentialities of microbial control methods under appropriate environmental conditions.

1880—Metchnikoff reiterated his beliefs in the use of fungi to control insects. He advocated the mass production of entomogenous fungi by artificial means for purposes of field distribution. Succeeded in propagating the green-muscardine fungus artificially.

1884—Isaak Krassilstschik organized a small laboratory for producing large quantities of *Metarrhizium* spores. Conducted field tests near Kieff.

1885—*Bacillus alvei*, the cause of European foulbrood of the honeybee, described by F. R. Cheshire and W. W. Cheyne.

1887–1898—Attempts by S. A. Forbes, of Illinois, and F. H. Snow, of Kansas, to control the chinch bug by means of the fungus *Beauveria globulifera* (Speg.). Other aspects of insect pathology were studied by Forbes during this period. Snow and Forbes responsible for stimulating the interests of American entomologists in the possibilities inherent in microbial control methods.

1888—The publication, by Roland Thaxter, of his monograph on the Entomophthoraceae of the United States; followed by his life work on the Laboulbeniaceae (1896–1931).

1892—M. C. Cooke published his book on entomogenous fungi.

1894–1898—G. Bolle correctly associated the polyhedral bodies, seen in silkworm jaundice, with the causative agent of the disease. He made the important observation that the polyhedral bodies are soluble in weak alkalis.

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